

Effect of phenolic compounds Extraction of Green tea (*Camellia sinensis L*) On the Glutathione of Alloxan Experimental Induced-Diabetic Rabbit.

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Abstract

Diabetes Mellitus is considered as a member of oxidative stress syndrome. It is associated with an imbalance between types of free radicals and scavenger's system. This study showed the effect of the polyphenol compounds extracted from green tea (*Camellia Sinesis L.*) on alloxan induced diabetic rabbits to determine their role in treatment of diabetes mellitus, their effect on enzymatic antioxidant and to know the histological effects on the diabetic kidney. The rabbits group was divided into five groups, each group consist of 10 rabbits. The blood and tissue samples from these groups were taken for analysis and the result were as follow:There was a significant increase in Glucose .when polyphenol extract of green tea was used gave a significant decrease in all parameters ($p<0.05$) with dose rate of 100mg/kg of body weigt,200mg/kg of body weight as compared with diabetic group. There was a significant decrease in glutathione. When polyphenol extract of green tea was used with dose rate 100mg /kg of body weight gave a significant increase ($p<0.05$) in all parameters as compared with diabetic group.In comparison between the efficacy of herbs polyphenol compound in green tea, glibeneclamide ,it was appeared that the herbs are the better based on efficacy on treatment of diabetes and their effect on glucose and glutathione. The present results revealed that alloxan was effectively induced diabetes by partial destruction of b-cells of pancreas which lead to elevation of blood glucose level. As a consequence of hyperglycemia the abnormal effect was obvious in certain tissues in the body which attributed to the effect of diabetes .Histological investigations shows that all the lesions in the kidney that result from diabetes such as hypertrophy, degeneration and hylanosis of the glomeruli.

تأثير مستخلص المركبات الفينولية من نبات الشاي الأخضر على الكلوتاتايون

وعلى داء السكري المحدث تجريبيا في الأرانب

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المستخلص

يعد مرض السكري من العناصر المتلازمة لضغط المؤكسدات ذات الصلة بالنوازل بين أنواع الأوكسجين الفعال ونظام الكاسحات. استهدف هذا البحث دراسة تأثير المركبات الفينولية المستخلصة من الشاي الأخضر *Camellia sinensis L.* في الأرانب المحدث بها السكري بواسطة الألوكان لمعرفة علاج داء السكر وتأثيرهما على الأنزيم الكلوتاتايون ولمعرفة التأثيرات النسيجية التي تطرأ على الكلية. شملت هذه الدراسة على خمسون من الأرانب وبعمر (6-12) شهرا وقسمت إلى خمس مجاميع كل مجموعة تضم 10 من الأرانب. تم أخذ العينات الدموية والنسيجية لفحصها وتحليلها وتم الحصول على النتائج الآتية: زيادة معنوية في الكلوكوز حيث أحدثت الخلاصة الفينولية للشاي الأخضر انخفاضا ملموسا إحصائيا ($p<0.05$) عندما استخدمت بتراكيز

٢٠٠ و ١٠٠ ملغم/كغم من وزن الجسم مقارنة بمجموعة السيطرة. بينما لوحظ نقصان معنوي في الأنزيم الكلوتاتايون حيث أحدثت الخلاصة الفينولية للشاي الأخضر ارتفاعاً ملموساً إحصائياً ($p < 0.05$) عندما استخدمت بتركيز ١٠٠ و ٢٠٠ ملغم من وزن الجسم مقارنة بمجموعة السيطرة. لذلك وبعد المقارنة بين فعالية الأعشاب مع الجلابينكلامايد تبين إن العشب أعلاه أكثر كفاءة في خفض سكر الدم وتأثيرها على الأنزيم الكلوتاتايون. أشارت النتائج إلى إن الألوكان يحدث مرض السكر، ويرفع مستوى سكر الدم من خلال تحطيمه الجزئي لخلايا بيتا في البنكرياس، ونتيجة لارتفاع السكر تظهر تغيرات نسيجية في الكلية المدروسة والتي عزيت إلى الإصابة بمرض السكر، والتي تتضمن وعلى سبيل المثال التضخم و الانحلال والتحول الزجاجي للكبيبات في الكلية.

Introduction

Diabetes is a disease characterized by insufficient secretion or improper functioning of insulin regulates the amount of blood sugar in our tissue. Damage caused by free radical is possible involved in B-cell destruction and in the pathogenesis of diabetes mellitus. Alteration of metabolic process in diabetes also influence enzymatic defenses, and these changes may be associated with late complications of diabetes^(1,2). Glutathione is γ -glutamyl cysteinyl glycine, the most abundant non-protein thiol compound in mammalian cells, is biosynthesized from amino acid precursors. Tissue glutathione plays a central role in antioxidant defense. The hydrogen atom of SH group abstraction is one of the most important aspects of GSH reactivity considering its function as an antioxidant. Glutathione as a product of glutathione reductase (GR) catalyzed to maintain the balance between GSH and GSSG. In fact, the ratio of reduced to oxidized GSH within cell is often used scientifically as a measure of cellular toxicity^(3,4). Many recent studies have found decrease GSH levels in different disease states especially those associated with oxidative stress like DM^(5,6). The traditional tea (*Camellia sinensis*) infusion is characterized by a high content of flavonoids. The active constituents of green tea contain volatile oils, vitamins, minerals, caffeine, and polyphenols particularly

the Catechins called epigallocatechin gallate (EGCG). Flavonoids are a large group of phenolic products of plant metabolism with a variety of phenolic structures that have unique biological properties and may be responsible for many of the health benefits attributed to tea. Many in vitro studies show that the flavonoids present in tea have strong antioxidant and metal-chelating properties and may therefore protect cells and tissues against free oxygen radicals. A large number of studies support the hypothesis that oxidative damage to DNA, lipids and proteins may contribute to the development of cardiovascular disease, cancer and neurodegenerative disease^(7,8). Reactive oxygen and nitrogen species are formed in the human body and endogenous antioxidant defenses are not always sufficient to counteract them completely. Diet-derived antioxidants may therefore be particularly important in protecting against chronic diseases. The flavonoids found in green and black tea are very effective antioxidants in vitro and may therefore be active as antioxidants in the body⁽⁸⁻¹⁰⁾. The aim of this study was to determine the role of phenolic compounds found in green tea in the treatment of diabetes mellitus, determine their effects on glutathione, and study their effect on diabetic kidney.

Materials and Methods

Fifty (50) healthy local adult rabbits of age ranged (6-12 months) and their body weight ranged (1.5 - 2) kg obtained from local market of

Baghdad city. Animals were divided into 5 groups each contains 10 animals, as follows:-

1. Control not treated (tap water).
2. Diabetic- non treated
3. Diabetic rabbits treated with extract of green tea (100mg/kg B.wt.).
4. Diabetic rabbits treated with extract of green tea (200mg/kg B.wt.).
5. Diabetic rabbits treated with glibenclamide (0.5mg /kg B.wt.).

Extraction of phenolic Compound

The leaf of green tea and grape seed (yellow seed) were dried and powdered, according to Gayon method⁽¹²⁾. 200 gram of plant powder was weight and added to 800ml of 2% acetic acid and extracted, the mixture was left for 24 hours in an incubator at 50C°, then filtered through filter paper to remove all the residual materials. The clear extracted solution treated with the same volumes of n- propanol, and then saturated with NaCl. The upper layer was separating by funnel, then dried at 45C° using an incubator. **Pilot study:** The dose used in this study was estimated according to the result of pilot study.

Induction of diabetes mellitus

Diabetes was induced in rabbits by injection of alloxan tetra hydrate at a dose of 180 mg /kg body weight IV In marginal ear vein⁽¹³⁾. Soon the animal were injected with 10 ml of 20% glucose solution S/C. Glucose solution 10% was given for 24 hours instead of the tap water in order to reduce alloxan hypoglycemic shock .

Tissue preparation for lig microscopy

The animals were survived for their end period and then sacrificed using high dose inhalation of chloroform inside glass box .The animals were dissected, ,the abdomen was opened by a longitudinal incision, and a few drops of the fixative were put on the kidneys

before they were dissected out within a few seconds. Then dissected kidneys and immersed in 10% formalin for 24h for fixation and histological tissue processing purposes . After fixation each kidney sample were cut with a blade to small piece . The specimens washed in running tap water, dehydrated through graded ethyl alcohol, 50, 70, 90, 100, half hour for each, and cleared by xylol. Then, embedded in melted paraffin inside paraffin bath at 64 C for half hour and blocked in paraffin wax. All sections then immersed in xylol for 10 minutes, rehydrated through graded alcohol 5-10 minutes for each, and finally imbedded inside, water for 3 minutes . Sections were stained using haematoxylin and eosin. Then placed in graded alcohol, 50, 70, 90, 100.

Biochemical Assay Methods

Glucose Estimation:-

Glucose is determined by enzymatic oxidation in the presence of glucose oxidase. The hydrogen peroxide formed reacts under catalysis of Peroxidase, with phenol and 4-aminophenazone to form a violet quinoneimine dye as indicator.

Determination of serum glutathione:-

5,5-Dithiobis (2-nitrobenzoic acid) (DTNB) is a disulfide chromogen that is readily reduced by sulfhydryl group of GSH to an intensely yellow compound. The absorbance of the reduced chromogen is measured at 412 nm and is directly proportional to the GSH concentration⁽¹⁴⁾.

Results

The effect of glibenclamide & green tea intake on glucose level in alloxan diabetic rabbit Table(1) showed obtained that the glucose activity is significantly increased in alloxan diabetic rabbits $P < 0.05$ (245 ± 22.8) mg/dl compared with (118 ± 9.249) mg/dl in control rabbit. The level of glucose were decreased in from

(245±22.8) mg/dl to (128±9.442) mg/dl after intake glibenclamide .After treatment with 100 mg of green tea a significant decreased in glucose level (143±7.41) mg/dl was observed compared with control group which indicated a positive correlation effect of green tea intake. The level of glucose was significantly reduced $P<0.05$ (132±6.82) mg/dl in alloxan diabetic rabbits receiving 200mg of green tea compared with the level of control group statistically 100, and 200 mg green tea for 20 weeks compared with Glibeneclamide were not significant as shown in table(1). *The effect of glibeneclamid& green tea intake on GHS level in alloxan diabetic rabbit* Table (1) showed obtained that the GHS levels is significantly increased in alloxan

diabetic rabbits $P<0.05$ (0.68±0.240) $\mu\text{mol/L}$ compared with (1.28±0.409) $\mu\text{mol/L}$ in control rabbit. The level of GHS were decreased in from (1.28±0.409) $\mu\text{mol/L}$ to (0.82±0.126) $\mu\text{mol/L}$ after intake glibenclamide. After treatment with 100 mg of green tea a significant decreased in GHS level (0.91±0.068) $\mu\text{mol/L}$ was observed compared with control group which indicated a positive correlation effect of green tea intake. The level of GHS was significantly reduced $P<0.05$ (0.92±0.067) $\mu\text{mol/L}$ in alloxan diabetic rabbits receiving 200mg of green tea compared with the level of control group statistically 100, and 200 mg green tea for 20 weeks compared with Glibeneclamide were not significant as shown in table(2).

Table(1): Means and standard deviations of Blood sugar levels in healthy and alloxan diabetic rabbits after twenty weeks of daily administration of glibenclamide (100mg/kg, and 200mg/kg), of green tea .

Group	Glucose (mg/dl)
Healthy rabbits	118±9.249
Alloxan diabetic rabbits	245±22.8
Alloxan diabetic rabbits after green tea 100mg intake	143±7.41
Alloxan diabetic rabbits after green tea 200mg intake	132±6.82
Alloxan diabetic rabbits after glibeneclamide intake	128±9.442

Significant compared with diabetic group

Table (2): Means and standard deviations of GHS levels in healthy and alloxan diabetic rabbits after twenty weeks of daily administration of glibenclamide (100mg/kg, and 200mg/kg), of green tea.

Group	GHS($\mu\text{mol/L}$)
Healthy rabbits	1.28 \pm 0.409
Alloxan diabetic rabbits	0.68 \pm 0.240
Alloxan diabetic rabbits after green tea 100mg intake	0.91 \pm 0.068
Alloxan diabetic rabbits after green tea 200mg intake	0.92 \pm 0.067
Alloxan diabetic rabbits after glibenclamide intake	0.82 \pm 0.126

Significant compared with diabetic group



Figure (1): Shrinkage of glomerulus in glibenclamide group after treatment. H&E.40X.

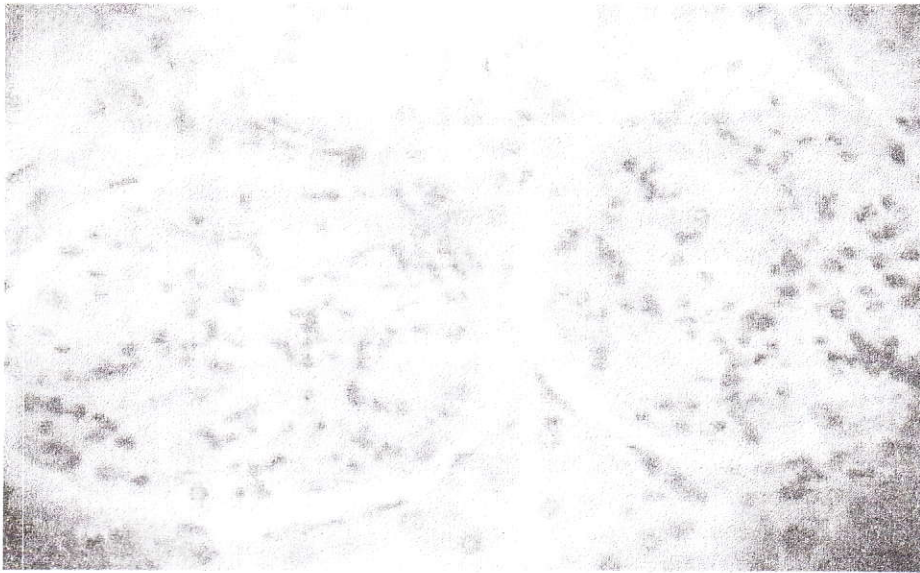


Figure (2): Hypertrophy of glomeruli in diabetic group. H&E.40X.

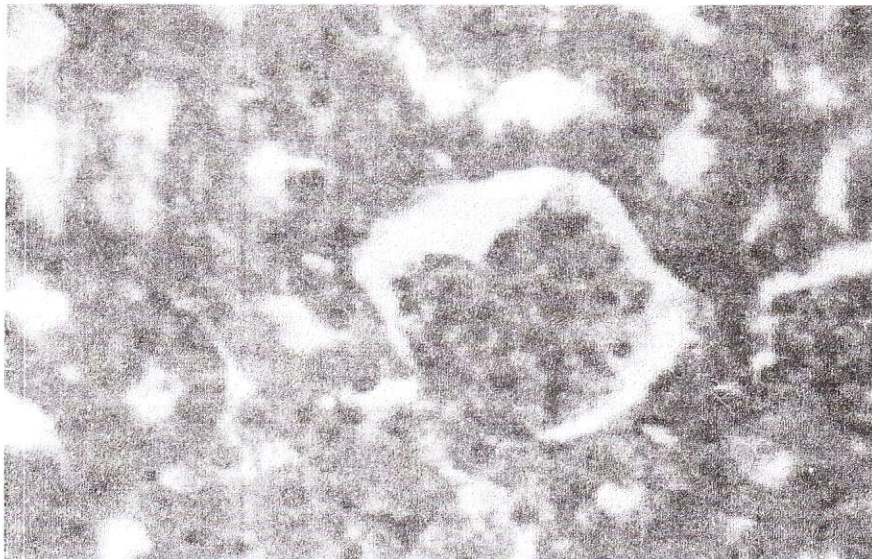


Figure (3): Glomeruli appear nearly normal, proximal convoluted tubules appear nearly normal, distal convoluted tubules appear nearly normal in green tea group after treatment. H&E.40X.

Discussion

Effect of polyphenol compound intake on blood glucose levels in alloxan induced diabetic rabbits. As shown in table (1) a significant decrease in the levels of blood sugar were obtained in alloxan induced diabetic rabbits

receiving polyphenol compound daily for 20 week compared with before treatment. The persistence of hyperglycemia in DM patients under study leads to an increase of oxidative stress by several mechanism including glucose authorization and non-

enzymatic protein glycation, as well as the same condition occurred in alloxan induced diabetic rabbits, so the positive effect of polyphenol compound may be investigated more details of more than one mechanism. Polyphenol compound act by decreasing insulin resistance at the level of muscle and fat, so that the body owns insulin becomes more efficient in its action to control blood glucose. Polyphenol compounds do not cause low blood glucose (hypoglycemia) but in combination with other agent such as glipizide. The peak blood glucose lowering effect is seen at 4hr underlying the insulin releasing action of polyphenol compound argue against an action similar to sulphonylurea drugs, currently used for diabetic therapy. These agents may be act by binding to sulphonylurea receptors resulting in closure of the membrane K-ATP channels, and elevation of intracellular calcium ions. Possible action of polyphenol compound may include enhancement of β - cell glucose metabolism or activation of enzyme systems generating cyclic AMP or phospholipids derived messengers. In conclusion, the antihyperglycemic action of polyphenol compound is associated with the stimulation of insulin secretion and improvement sensitivity of insulin⁽¹⁵⁾.

Effect of polyphenol compound intake on GHS levels in alloxan induced diabetic rabbits.

The levels of GHS in alloxan induced diabetic rabbits were significantly increased receiving polyphenol compound daily for 20 week compared with before treatment. Serum GHS plays a central role in antioxidant defense. GHS detoxifies ROS such as H₂O₂ and lipid peroxides directly or in a glutathione peroxidase (GPX) catalyzed mechanism. GHS also generates the major aqueous and lipid phase antioxidant, ascorbate and α -

tocopherol^(3,16,17). In DM GHS glutathione deficiency resulting from NADPH is used in the polyol pathway where glucose is reduced to sorbitol by aldose reductase. Increased activity in this pathway causes a depletion of reduced GHS, which may weaken the antioxidant defense^(7,18).

Microscopic investigation

In diabetes mellitus frequently damage the kidney, leading to impaired excretory and homeostatic function. Decreased blood flow through the glomerular capillary system because of the thickening of the arteriole and arteriolar walls, and the consequent reduction in the Lumina of this vessels, produces chronic ischemia of the tubular system and reduces glomerular filtration if prolonged, this lead to disuse shrinkage of the compounds of the glomerulus (glomerular hyalinization) and atrophy of the tubules. When this changes affected most of the glomeruli and their associated tubular system all of the functions of the kidney are impaired. A good glycemic control may help in the prevention of glomerular hypertrophy and preventing many lesions that accompanied diabetic nephropathy, this was in according with Ballard *et al*⁽¹⁹⁾, and Vasquez *et al*⁽²⁰⁾. Glomerular hypertrophy and mesangial expansion which are typical changes in diabetic nephropathy^(21,21). Factors contributing to glomerular enlargement in diabetic nephropathy include increased intraglomerular pressure, growth of glomerular cells, and accumulation of extracellular matrix⁽²³⁾. The present study shows that phenolic compounds especially in 200mg of green tea, have most positive results in preventing many lesions that accompanied diabetic nephropathy.

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